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Steady-state indirect bilirubin level in sickle cell anemia: A comparative hospital-based cross-sectional study

Modibo Coulibaly [1,*], Eusèbe Adia Amdjambri [2], Aboubacar Sidiki Traoré [3], Moussa Diawara [4] and Ibrahima Sanogo [2]

[1] *Department of Biomedical Laboratory, Sominé DOLO Hospital of Mopti, Mopti, Mali.*
[2] *Department of Hematobiology, Teaching Hospital of Yopougon, Abidjan, Côte d'Ivoire.*
[3] *Department of Medicine, Sominé DOLO Hospital of Mopti, Mopti, Mali.*
[4] *Department of Biomedical Laboratory, Military Hospital/Infirmary of Kati, Koulikoro, Mali.*

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Abstract

Background: The major forms of sickle cell disease are characterized by hemolysis. The extent of this hemolysis may itself depend on the form of the disease even in the steady-state. This study aims to compare the indirect bilirubin level between the homozygous and beta plus sickle cell anemia ($SAFA_2$) in the steady-state conditions.

Material and methods: Fifty subjects of each forms, homozygous and beta plus sickle cell anemia were enrolled in a comparative hospital-based cross-sectional study from April 2008 to May 2008 at the laboratory of the university hospital of Yopougon, Ivory Coast. Subjects of each form awaiting their visits were selected from the database of the patients regularly followed in the clinical hematology department of Yopougon University Hospital. During their visits, clinical and socio-demographic data were collected, and a blood sample was also taken to carry out biological examinations. Blood count and bilirubin testing were performed by using Sysmex Kx-21™ and BioMérieux KONELAB-20™, respectively. Data were analysis in the software Statistical Product and Service Solutions version 12.0. Shapiro test was used to verify data normality and Student t-test for the comparison of parametric data means or Mann-Whitney's independent test for none-parametric data. Pearson Chi square tests or Yate's correction test for continuity where the first test was not appropriate. The threshold of statistical significance was set at $p \leq 0.05$.

Results: The median Hb was higher in $SAFA_2$ patients compared to homozygous $SSFA_2$ patients 10.7 g/dL [IQR = 8] vs 7.3 g/dL [IQR = 7]; $p < 0.001$. In contrast, the median of indirect bilirubin was lower in $SAFA_2$ patients compared to $SSFA_2$ patients 5.6 µmol/L [IQR = 10] vs 15.1 µmol/L [IQR = 13], $p < 0.001$. The ratio of these two medians shows that subjects $SAFA_2$ hemolysis 2.7 times less than the homozygous subjects $SSFA_2$ in the steady-state. Out of the one hundred subjects, indirect hyperbilirubinemia defined as indirect bilirubin median > 14 µmol/L was higher in men than in women 79.2% vs 20.8%, $p = 0.01$ whereas patients age groups was not associated to indirect hyperbilirubinemia, $p = 0.4$.

Conclusion: Our data suggest that the hemolysis is higher in subjects $SSFA_2$ than $SAFA_2$ subjects in the steady-state. This marked chronic hemolysis of $SSFA_2$ subjects must be taken into account when it comes to give a comprehensive care to these subjects.

Keywords: Sickle cell anemia; Steady-state; Indirect bilirubin; Hemolysis.

* Corresponding author: Modibo COULIBALY
Department of Biomedical Laboratory, Sominé DOLO Hospital of Mopti, Mopti/Mali.

1. Introduction

Sickle cell disease (SCD) is a major inherited red blood cell disorder that affects hemoglobin (Hb), the protein that plays oxygen-carrying function through the organism. Despite major advances in the areas of the pathogenesis and the management of congenital hemoglobinopathies, SCD remain a public health problem in somewhere between the 15th parallel of north latitude and the 20th parallel of South latitude called "Lehmann's Sicklemic Belt" [1]. It is estimated that 300 millions of people will carry the sickle cell trait, 6,400,000 peoples will suffer from SCD and 300,000 children will born with the disease each year from 2010 to 2050 if nothing is done [2-3]. SCD patients are prone to chronic hemolysis even in the steady-state and therefore an increase in their total bilirubin (TB) and indirect bilirubin (IB) levels and decrease of their Hb level. The extent of this chronic hemolysis is very variable according to the SDC forms [4-5]. This great heterogeneity of the clinical and biological phenotypes of SCD is of great importance and should be taken in account when it happen to manage SCD patients in acute-phase, mainly in it hepatobiliary manifestations [6]. The aim of this work was to describe the hemolytic profile of Sß$^{+}$ thalassemia ($SAFA_2$) patients compared to homozygous $SSFA_2$ patients in the steady-state.

2. Material and methods

Fifty subjects of each forms, $SSFA_2$ and $SAFA_2$ were enrolled in a comparative hospital-based cross-sectional study from April 2008 to May 2008 at the department of clinical hematology of the university hospital of Yopougon, Ivory Coast. Subjects in steady-state of each form awaiting their visits were selected from the database of patients regularly followed. Patients received during their visits were recruited consecutively according to their hemoglobin profile which was already determined by agarose gel electrophoresis in alkaline medium combined with isoelectrofocusing and recorded in their case report forms. The steady-state was defined as the absence of complications and acute manifestation of sickle cell disease in the 15 days preceding patients visits at the physician office. Clinical and sociodemographic data were recorded for all subject by the physicians. Venous whole blood were collected in an EDTA and in a dry tubes for total blood count and biochemistry analysis purpose for each fasting patient. The blood count was performed by Sysmex Kx-21™ analyzer whereas the biochemical parameters, direct bilirubinemia and total bilirubinemia were made on KONELAB-20™ analyzer. Data were captured in Microsoft Excel® version 2.0 and exported to Statistical Product and Service Solutions (SPSS) version 23.0 (IBM Corp. 2015, Armonk, NY) software for statistical analysis. Descriptive statistics were presented as mean values ± standard deviation (SD) or medians with interquartile ranges (IQRs) for none normal continuous variables, and proportions (as percentages) for categorical variables. Tables and graphical representations were used to summarize the data. Statistical associations of dependent and independent variables were assessed using Chi square tests or Yate's correction test for continuity where Chi square test was not appropriate. All tests were 2-tailed and *p-values* of less than 0.05 was considered statistically significant.

3. Results

Out of the 100 patients enrolled, 54 were men (54%) and 46 were women (46%). The sex-ratio was 1.2 (Figure 1). The average age was 17.9 ± 5.3 years with the extremes ranging from 2 to 61 years (Figure 2). The age group 0-15 years was the most represented in our series 51%. Patients with no apparent clinical signs accounted for 85% whereas the paleness was found in 10% of patients (Table 1). The Hb median in Sß$^{+}$ patients ($SAFA_2$) was 10.7 g/dL [IQR = 8] whereas the Hb median in homozygous patients ($SSFA_2$) was 7.3 g/dL [IQR = 7] (Table 2). Indirect bilirubin median was 5.6 µmol/L [IQR = 10] for $SAFA_2$ subjects and 15.1 µmol/L [IQR = 13] for $SSFA_2$ subjects (Table 2). Hb median was higher in $SAFA_2$ than in $SSFA_2$, 10.7 g/dL [IQR = 8] vs 7.3 g/dL [IQR = 7], $p < 0.001$. The median of total bilirubin was higher in $SSFA_2$ subjects compared to $SAFA_2$ subjects, 20.7 µmol/L [IQR = 23] *versus* 8.6 µmol/L [IQR = 12], $p < 0.001$ (Table 2). Also, the median of indirect bilirubin was lower in $SAFA_2$ subjects compared to $SSFA_2$ subjects 5.6 µmol/L [IQR = 10] *versus* 15.1 µmol/L [IQR = 12], $p < 0.001$ (Table 2). Indirect bilirubin accounted for 73% and 64% of total bilirubin in $SSFA_2$ and $SAFA_2$ patients, respectively. The ratio of indirect bilirubin medians shows that $SAFA_2$ subjects were 2.7 times less prone to hemolysis than $SSFA_2$ subjects in the steady-state. Out of the one hundred subjects, indirect hyperbilirubinemia defined as indirect bilirubin median > 14 µmol/L was higher in men than women 79.2% vs 20.8%, $p = 0.01$ (Table 3) whereas patients age groups was not associated to indirect hyperbilirubinemia, $p = 0.4$ (Table 4).

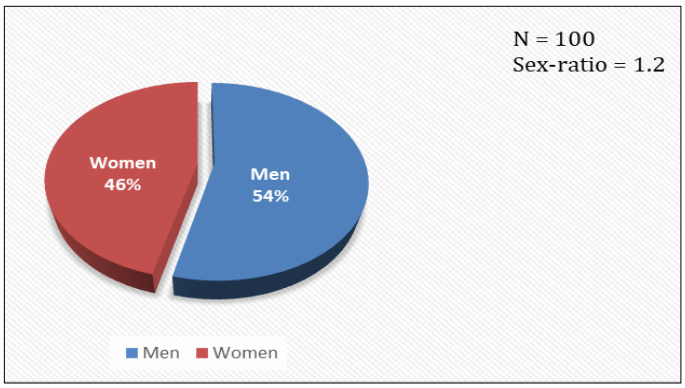

Figure 1 Distribution of patients by sex

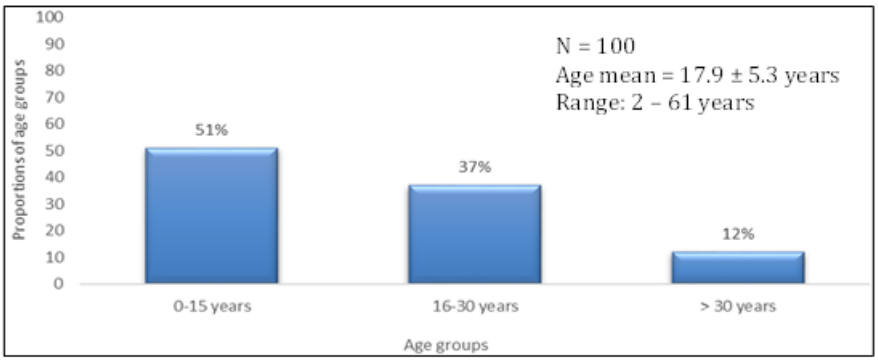

Figure 2 Distribution of patients by age groups

Table 1 Comparison of clinical signs between Hb-SSFA2 and Hb-SAFA2

Clinical signs	Hb-SSFA2		Hb-SAFA2		p
	n	%	n	%	
No clinical sign	4	8	24	48	< 0.001
Splenomegaly	11	22	6	12	0.3
Paleness	35	70	20	40	0.01
Total	50	100	50	100	

Table 2 Comparison of Hb, TB and IB levels between Hb-SAFA2 and Hb-SSFA2 groups

Biological parameters/Sickle cell form		Q1	Median	Q3	*p*
Hb g/dL	Hb-SAFA2	4	10	12	< 0.001
	Hb-SSFA2	2	7	9	< 0.001
TB µmol /L	Hb-SAFA2	6	9	18	< 0.001
	Hb-SSFA2	12	21	35	< 0.001
IB µmol/L	Hb-SAFA2	2	6	12	< 0.001
	Hb-SSFA2	9	15	22	< 0.001

TB = Total bilirubin, IB = Indirect bilirubin.

Table 3 Variation of indirect bilirubin level according to patient's sex

Sex	Normal*		High **		p
	N	%	N	%	
Male	35	46.1	19	79.2	
Female	41	53.9	5	20.8	0.01
Total	76	100	24	100	

*Indirect bilirubin median ≤ 14 µmol/L; **Indirect bilirubin > 14 µmol/L

Table 4 Variation of Indirect bilirubin level according to patient's age groups

Age groups (years)	Variation of indirect bilirubin				p
	Normal*		High**		
	N	%	N	%	
2-15	39	51.3	12	50	
16-30	26	34.2	11	45.8	0.4
> 30	11	14.5	1	4.2	
Total	76	100	24	100	

4. Discussion

The aim of our work was to assess the extent of the chronic hemolysis between homozygous $SSFA_2$ subjects compared to Sß$^+$ thalassemia $SAFA_2$ subjects in the steady-state in a cross-sectional hospital-based study. Our study was conducted at the department of clinical hematology of the university hospital of Yopougon, Ivory Coast. The case were enrolled from January 2008 to March 2008. At this period, malaria transmission is low and therefore interfere less with SCD hemolysis [7]. Although the transmission of sickle cell anemia is not sex-linked, we found a sex-ratio of 1.2. Also, Tolo A et *al.,* 2006, Sekongo YM et *al.,* 2015 each reported a sex-ratio of 1.3 in major sickle cell patients at the department of hematology of Yopougon teaching hospital and at the Abidjan blood transfusion center, respectively [8-9]. In contrast, Nanitelamio E et *al.*, 2021 found a different sex-ratio of 0.9 in Congo Brazzaville in a similar study on hematological and biological profiles in sickle cell patients [10]. The average age was 17.9 ± 5.3 years and the extremes varied from 2 to 61 years. This result is different from that obtained by Tolo A et *al., [*8] who found age extremes of 2 to 38 years with an average age of 14.6 years. Tolo series concerned exclusively $SSFA_2$ subjects which may have a shorter life expectancy than the Sß$^+$ subjects. This short life expectancy could be explained by the high morbidity and mortality of $SSFA_2$ subjects due to their vulnerability to infections [11]. Patients with no apparent clinical signs accounted for 85% of the study population. This was related to the fact that our study involved only apparent steady-state patients. We found a pallor in 10% of patients. The rest of the signs was little represented and consisted of slight splenomegaly. A comparison study in Niger performed by Mounkaila B et *al*., 2015 showed that pallor, splenomegaly and hepatomegaly were found more in $SSFA_2$ subjects compared to SC subjects [12]. The median of hemoglobin (Hb) level was significantly lower in $SSFA_2$ subjects compared to $SAFA_2$ subjects: 7 g/dL [IQR = 7] *versus* 10g/dL [IQR = 8], $p < 0.001$. Several studies have found a significant difference between the Hb levels of the homozygous subject compared to other forms of sickle cell disease in the steady-state, thus demonstrating greater chronic hemolysis in homozygous subjects [10-13]. Total bilirubin (TB) and indirect bilirubin (IB) were significantly lower in Sß$^+$ thalassemic ($SAFA_2$) subjects than in $SSFA_2$ subjects 9 µmol/L [IQR = 12] *versus* 21 µmol/L [IQR = 23]; 6 µmol/L [IQR = 10] *versus* 15 µmol/L [IQR = 13], respectively all $p < 0.001$. This low level of TB and IB highlight the hypothesis of lower hemolysis in $SAFA_2$ subjects compared to $SSFA_2$ subjects. Our results are in line of that reported by Mounkaila B et *al*., who also found a lower level of IB (7 mg/L) in SC subjects compared to homozygous $SSFA_2$ subjects (15.8 mg/L) [12]. Aminu SM et *al*., 2017 in a control case study reported higher TB in $SSFA_2$ subjects compared to control subjects Hb-AA 31.80 ± 25.17 µmol/L versus 18.73 ± 6.34 µmol/L [14]. Pandey S et *al*., found a higher TB in $SSFA_2$ homozygous subjects compared to Sß^0 thalassemic subjects (SFA_2) 3.2 ± 1.3 mg/L *versus* 2.5 ± 1.4 mg/L. Nanitelamio E et *al*., reported a lower level of direct bilirubin (DB) mean than that of our series in homozygous subjects of 12.7 ± 2.6 µmol/L versus 15.1± 9.8 µmol/L [15]. Dubert M et *al*., reported a higher TB in

$SSFA_2$ subjects compared to Sß⁺ subjects ($SAFA_2$) 27.0 µmol/L [IQR = 21] versus 11.0 µmol/L [IQR = 13] [13]. In our series, the ratio of IB median in Sß⁺ thalassemic subjects ($SAFA_2$) to $SSFA_2$ subjects (15.13/5.58) shows that the subjects $SAFA_2$ hemolysis 2.7 times less than the $SSFA_2$ subjects. For the investigation of the relationship between IB, socio-demographic and clinical parameters, we divided our study population into two groups. A first group consists of patients with IB ≤ 14 µmol/L and the second group representing patients with IB > 14 µmol/L. The change in the median of IB was significantly associated with the sex of the patients 19/24 (79.2%) *versus* 5/24 (20.9%), $p = 0.05$. This difference could be explained by the intense physical activity of men, which could promote hemolysis and thereby increase in IB. Hyper IB was frequent in young subjects. This could be explained by the high mortality of this disease before advanced age. No difference was observed between the ethnic groups $p = 0.45$ suggesting the same pattern of subjects exposition to the disease.

According to Hamad Z et *al*., 2013 [16] and Batista JVGF et *al*., 2021 [17], sickle cell anemia patients experience hyperbilirubinemia as a result of enhanced erythrocyte destruction. This could lead to cholelithiasis development in a subset of patients. Evidence suggests that hyperbilirubinemia may be related to genetic variations, such as the UGT1A1 gene promoter polymorphism.

Our study has shown that sickle cell disease is characterized by chronic hemolysis even in the steady-state. However, it did not include cases of control and cases in a crisis situation, which could give added value to the data obtained as indicated in the study of Nanitelamio E et al., 2021 [10].

5. Conclusion

Our data suggest that the hemolysis is higher in subjects $SSFA_2$ than $SAFA_2$ subjects in the steady-state. This marked chronic hemolysis of $SSFA_2$ subjects must be taken into account in the event of a crisis.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

Statement of informed consent

Each participant gave fully verbal consent prior to enrollment. The research protocol was reviewed and approved by the head of the department of hematology.

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